Distribution of Polybrominated Biphenyls after Dietary Exposure in Pregnant and Lactating Rats and Their Offspring

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Female rats were fed PBBs in the diet (50 ppm) from day 8 of gestation to day 21 of gestation, from day 1 postpartum to day 14 postpartum or from day 8 of gestation through day 14 postpartum. Levels of PBBs were measured in various tissues. Small concentrations of PBBs (<5 $\mu g/g$) were found in the brain, heart, lung, liver, small intestine, placenta, and gravid uterus. Larger concentrations (<30 $\mu g/g$) were found in kidneys, the nongravid uterus, skin, mammary tissue, and fat. Lactation did not significantly alter the concentrations of PBBs found in tissues other than mammary tissue.

Offspring were subjected to several exposure regimens by cross-fostering. Concentrations of PBBs in the neonatal livers were higher than in the adults nursing them. Transfer of PBBs via the milk appears to be much more important to appearance of PBBs in newborns than does placental transfer.

Introduction

In 1973 a flame retardant, FireMaster BP-6, was accidentally mixed with livestock feed in Michigan. FireMaster BP-6 is a mixture of polybrominated biphenyls (PBBs), and its introduction into livestock feeds resulted in substantial losses in the Michigan agricultural industry (1).

The PBBs appear to be similar to the polychlorinated biphenyls with respect to their chemical and biological stability (2–5). Because of the probability that PBBs will remain in the environment for quite some time, it is important to assess their effects on maternal, fetal, and neonatal animals. Some of these effects have been studied and are reported elsewhere (6). Important to the correlation of exposure to PBBs and their effects is a thorough examination of the distribution of PBBs in the body. This publication describes the tissue distribution of PBBs in pregnant and lactating rats and their offspring.

Materials and Methods

Timed pregnant Sprague-Dawley rats were obtained on day 5 of gestation (Spartan Farms, Haslett, Mich.). On day 8 of gestation these rats were randomly assigned to diets containing 0 or 50 ppm PBBs. FireMaster BP-6 was the form of PBBs used. This substance is a mixture of PBBs of which 2,2', 4,4',5,5'-hexabromobiphenyl comprises about 70% (2). The PBBs were dissolved in acetone and thoroughly mixed with ground food pellets (Wayne Lab Blox). The control diet was ground food pellets to which only acetone had been added.

Four treatment groups of maternal animals arose from this design: (1) animals which had never had dietary exposure to PBBs (control group), (2) those animals which received PBBs in the diet beginning on day 8 of gestation and which were sacrificed on day 21 of gestation (group A), (3) those animals which received PBBs from the day young were de-

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livered until sacrifice 14 days later (group B), and (4) those animals which received PBBs from day 8 of gestation through day 14 postpartum (group C).

Several groups of pups were obtained from the design. Pups in Group I were obtained by Cesarean section on day 21 of gestation from dams which had received PBBs in the diet beginning on day 8 of gestation. These pups were sacrificed immediately upon removal from the mothers.

The pups in group II were delivered normally by dams which had received PBBs in the diet from day 8 of gestation. These pups were cross-fostered at birth to dams which had received no PBBs. The pups were sacrificed on day 14 postpartum.Pups in group III were delivered normally by dams which had received no PBBs and were cross-fostered at birth to animals which had received PBBs in the diet since day 8 of gestation. The pups were sacrificed on day 14 postpartum.

Group IV was comprised of pups delivered normally by dams which had received no PBBs. They were cross-fostered at birth to dams which began to receive PBBs in the diet on the day cross-fostering occurred.

Pups in group V were born to mothers which had received PBBs in the diet from day 8 of gestation and were cross-fostered to animals which had also received PBBs in the diet from day 8 of gestation.

Foster mothers to groups III, IV, and V continued to receive PBBs in the diet until they and the pups were sacrificed on day 14 postpartum. All litters were normalized to 10 pups at birth.

For purposes of simplicity data from all control pups, whether sacrificed at cesarean section or 14 days postpartum were pooled. Only traces of PBBs were found in these animals regardless of age.

Animals were sacrificed by decapitation (pups) or cervical dislocation (adults). Tissues of interest were removed, weighed and homogenized with water. Maternal tissues selected for study were brain, heart, lung, liver, small intestine (duodenum), kidney, mammary gland (abdominal and inguinal), uterus, fat (abdominal), skin (removed from the lower back), and placenta. Tissues from young rats used were the liver and the complete gastrointestinal tract (excluding esophagus); levels of PBBs in the remainder of the carcass were also determined.

Extraction procedures for PBBs were based on those reported by others (7). Homogenates of maternal fat, mammary gland, and skin and neonate gastrointestinal tract were mixed with 5 volumes of petroleum ether (bp 40–60°C, redistilled before use). The phases were separated and the petroleum ether layer was reduced in volume to 5 ml. A 20-ml volume of acetonitrile saturated with petroleum ether

was then added. The phases were separated and the petroleum ether layer was discarded. Crystalline NaCl (ca. 50 mg) was added to the acetonitrile. PBBs were extracted from the acetonitrile with three 10-ml portions of petroleum ether. The petroleum ether fractions were combined, reduced in volume to ca. 2 ml and placed on a column of Florisil (5 mm × 100 mm). PPs were eluted with 3 ml of 6% diethyl ether in petroleum ether. The column eluate was evaporated to dryness and reconstituted in petroleum ether (50–100 μl) for mass fragmentography.

Homogenates of tissues other than those listed in the preceding paragraph were handled in a similar fashion, except that no acetonitrile partition step was necessary.

Stock standards were prepared in petroleum ether from the same substance fed to the animals, FireMaster BP-6. This substance is a mixture of PBB congeners; the most abundant congener (70%) is 2,2',4,4',5,5'-hexabromobiphenyl. Standard curves were extracted from water by the appropriate procedure (see above) and run at the same time as the samples. All glassware was treated with dimethyldichlorosilane prior to use.

Quantitation of PBBs was accomplished by mass fragmentography. Ions monitored were $m/e = 549$, the major ion in the M-79 cluster of 2.2',4,4',5,5'-hexabromobiphenyl, and $m/e = 628$, the major ion in the parent ion cluster of 2,2',4,4',5,5'-hexabromobiphenyl. The instrument used was a Finnegan 3200E mass spectrometer with PROMIM attachment; the ion source was operated at 70 eV and 500 μA. The gas chromatograph was fitted with a 1.7-m column packed with 1% OV-1. Column temperature was 240°C.

Data were analyzed by analysis of variance followed by Student-Neuman, Kuehls test (8). The level of significance was $p < 0.05$.

Results

Maternal brain, heart, lung, liver, small intestine, placenta, and the gravid uterus all contained less than 5 μg PBBs/g wet weight tissue (Table 1). Few statistically significant differences in levels of PBBs in the above tissues were demonstrable regardless of doubling the time on the diet (groups A and B versus Group C) and regardless of whether exposure was pre- or postpartum (group A versus group B).

Kidneys of exposed animals contained approximately 30 μg PBBs/g regardless of duration of exposure.

Mammary glands of exposed animals contained high levels of PBBs. Levels in nonlactating mam-

Tissue	Control	PBB level, μg/g wet weight tissue[b]		
		Group A	Group B	Group C
Brain	0.1 ± 0.1	0.8 ± 0.1^c	1.7 ± 0.2^c	1.2 ± 0.5^c
Heart	0.1 ± 0.0^d	0.5 ± 0.1^d	1.8 ± 0.6	1.6 ± 0.3
Lung	0.0 ± 0.0	2.4 ± 0.6^e	3.3 ± 0.6^e	5.0 ± 0.6
Liver	0.0 ± 0.0	4.2 ± 0.3^f	2.8 ± 1.2^f	4.0 ± 1.1^f
Small intestine	0.0 ± 0.0	0.5 ± 0.2^g	0.2 ± 0.0^g	0.2 ± 0.1^g
Kidney	0.0 ± 0.0	29.9 ± 7.5^h	33.0^i	30.5 ± 6.6^h
Mammary gland	0.4 ± 0.2	317.5 ± 25.4	65.2 ± 38.6^j	117.3 ± 40.5^j
Uterus	0.1 ± 0.0^k	0.3 ± 0.0^k	18.7 ± 5.8	33.6 ± 6.2
Fat	0.9 ± 0.6	330.0 ± 99.5^m	250.7 ± 83.2^m	502.7 ± 162.4^m
Skin	0.0 ± 0.0	22.4 ± 4.0^n	40.0 ± 6.7^n	175.9 ± 38.3
Placenta	0.0 ± 0.0	0.4 ± 0.1	not available for analysis	

[a] Group A received PBBs in the diet (50 ppm) from day 8 of gestation until sacrifice on day 21 of gestation. Group B received PBBs in the diet (50 ppm) from delivery until sacrifice on day 14 postpartum. Group C received PBBs in the diet (50 ppm) from day 8 of gestation until sacrifice 14 days after delivery.

[b] Values are means $\pm$ S.E.M. of 4–11 animals. Data for controls in each experiment were combined. Values with same superscript are not statistically different, $p < 0.05$.

[i] Single determination only.

mary tissue (group A) were significantly higher than in lactating mammary tissue (groups B and C).

The nongravid uterus (groups B and C) contained levels of PPBs which were significantly higher than those found in the gravid uterus (group A). Levels of PBBs in this organ increased with increasing duration of dietary exposure in lactating animals.

The highest tissue levels of PBBs were found in the fat. Large interanimal variation resulted in a failure to demonstrate statistically significant differences among exposed groups. Fat levels of PBBs in lactating animals exposed for 14 days (group B) tended to be lower than those exposed during both pregnancy and lactation (group C). Levels of PBBs in the fat of animals exposed only during pregnancy (group A) fell between the other two groups.

The skin of exposed animals contained significant levels of PBBs. Concentrations of PBBs in skin of animals exposed for 28 days (group C) were significantly higher than those in skin of animals exposed for only 14 days (groups A and B).

Concentrations of PBBs in tissues of animals born to and/or nursed by dams fed PBBs were highly variable (Table 2). The statistical tests used gave significant ($p < 0.05$) F ratios for treatment effects, but the large variability did not allow individual means to be declared different from one another. Levels of PBBs found in young animals exposed via the milk (groups III, IV, and V) tended to be greater than those in young animals exposed only *in utero* (groups I and II). Levels of PBBs in livers of young animals exposed via the milk (groups III, IV, and V, Table 2) tended to be higher than levels of PBBs in the livers of the adults nursing them (groups B and C, Table 1).

Discussion

The tissue distribution of PBBs in maternal animals followed a pattern similar to that of polychlorinated biphenyls (PCBs) (9, 10). Levels of PBBs were highest in those tissues with the highest lipid content such as mammary tissue and adipose tissue. PBBs, like PCBs (10), are accumulated to a much greater extent in mammary tissue of pregnant animals than of lactating animals. Takagi et al. (10)

Table 2. Tissue levels of PBBs in offspring of dams fed PBBs in the diet.[a]

Tissue	Control	PBB level, μg/g wet weight tissue[b]				
		Group I	Group II	Group III	Group IV	Group V
Liver	0.0 ± 0.5	0.2 ± 0.0	1.1 ± 0.7	16.1 ± 8.5	22.3 ± 15.2	9.5 ± 2.8
GI tract	0.3 ± 0.3	0.1 ± 0.0	1.4 ± 0.6	90.9 ± 58.5	68.0 ± 47.4	73.9 ± 34.1
Carcass	0.3 ± 0.3	1.6 ± 0.4	5.4 ± 2.3	132.7 ± 84.3	68.6 ± 34.8	149.7 ± 84.2

[a] Group I: pups from mothers fed 50 ppm PBBs in the diet, tissues analyzed on day 21 of gestation; group II: pups 14 days of age born to mothers fed 50 ppm PBBs in the diet but nursed by control dams; group III: pups 14 days of age born to control dams but nursed by dams which were fed 50 ppm PBBs in the diet from day 8 of gestation through day 14 postpartum; group IV: pups 14 days of age born to control dams but nursed by mothers fed 50 ppm PBBs in the diet from day 1 through day 14 postpartum; group V: pups 14 days of age born to and nursed by mothers which received 50 ppm PBBs from day 8 of gestation through day 14 postpartum.

[b] Values are means $\pm$ S.E.M. of 4–11 experimental units. Data from the control animals in each experiment were combined.

reported marked differences between lactating and pregnant rats in concentrations of PCBs in tissues other than mammary glands. No such marked differences were found for PBBs in this study. This may represent a real difference between PBBs and PCBs in the importance of milk for excretion, or it may be due to differences in experimental design. Takagi et al. (*10*) administered a single oral dose of PCBs while PBBs were fed in the diet in the study reported here.

Levels of PBBs in tissues other than fat, mammary tissue, skin, lung, and uterus are not elevated in animals fed PBBs throughout the pregnancy and lactation (group C) over those in animals receiving PBBs only during lactation (group B). More work is required to determine if plateau levels of PBBs have been reached in brain, liver, heart, small intestine, and kidney within 14 days of feeding.

Takagi et al. (*10*) showed that liver levels of PCBs in the offspring of treated dams were somewhat higher than liver levels in the mothers. This appears to be the case for PBBs as well, at least when the offspring are exposed via the milk. It has been reported that the hepatic mixed function oxidase system in nursing animals is stimulated when the dams are fed 1 ppm PBBs in the diet, while stimulation of the maternal hepatic mixed function oxidase system is not demonstrable at that level of PBBs in the diet (*11*). The data presented here suggest that since neonate hepatic levels of PBBs are higher than maternal levels, there may not be marked differences in sensitivity to stimulation by PBBs between the neonate and adult hepatic mixed function oxidase system. Dent et al. (*6*) have shown that aryl hydrocarbon hydroxylase (AHH) and epoxide hydratase (EH) activities in maternal liver are elevated as much or more than the same activities in neonate liver by exposure to PBBs.

The data presented here suggest that transfer of PBBs to the young via the milk is far more important than placental transfer (compare groups I and II with groups III, IV, and V in Table 2). This importance of transfer via the milk is underscored by comparing the body burdens of PBBs in groups I and II in Table 2. Body burdens of PBBs are roughly 9 μg for group I and roughly 113 μg for Group II. Since these groups were ideally exposed only transplacentally one would expect group I to have a body burden equal to or higher than group II. The reason group II has a much higher body burden

than group I is most likely due to the fact that the animals of group II spent some time (<4 hr) with their natural mothers before being cross-fostered to dams which had never received any PBBs. Intake of PBBs via the milk during this time may have accounted for the much larger body burden of PBBs in group 2.

The data presented here demonstrate that distribution of PBBs in pregnant and lactating rats bears some resemblance to the distribution of PCBs. Direct comparisons with pure isomers of each of the polychlorinated biphenyls are necessary to determine how close that resemblance is. Furthermore, the data suggest that the most important mechanism of transfer of PBBs from mother to young is not via the placenta but via the milk.

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